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TRANSMISSION OF MALARIAL FEVER IN THE CANAL ZONE BY ANOPHELES MOSQUITOES *

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ANCON, CANAL ZONE

It is between eleven and twelve years since Ronald Ross conducted his famous experiments demonstrating the transmission of malarial fever by mosquitoes. This demonstration, it seems to me, has contributed more to the forging of the key to the control of the tropics than any other one in the annals of tropical medicine, for Ross' discovery focused the attention of the medical world on the part played by suctorial invertebrates in the transmission of infectious diseases. Knowledge of this doctrine of the insect intermediary host, coupled with Dr. H. R. Carter's invaluable observation of the extrinsic period of incubation, enabled the yellow fever commission to verify Finlay's notion about the transmission of yellow fever by stegomyia mosquitoes.

The application of these two discoveries, Ross' and Reed's, to the problems of preventive medicine by your President-elect has made yellow fever a historic disease in Panama and is surely reducing the morbidity and mortality from malarial fever to the vanishing-point.

The object of the work briefly reported here was to go over the whole subject of the transmission of malarial fever by anopheles mosquitoes. I have been guided by the work of Ronald Ross, Stephens, Christophers and James, whose investigations of malaria in India and Africa must stand as models for all future workers on this subject. In every malarial region it is a matter of importance to determine the varieties of anopheles in that region, their bionomics, the species hospitable to malaria and those which can not transmit it. The

* Read in the Section on Practice of Medicine of the American Medical Association, at the Sixtieth Annual Session, held at Atlantic City, June, 1909.

English investigators, James, Christophers and Stevens, observed that different species of anopheles had different degrees of susceptibility to malaria; certain species were natural transmitters of malarial fever, while others were rarely, if ever, found infected naturally, although it would be possible to infect them under laboratory conditions. It is important, then, to determine what varieties of anopheles are present and which ones may act as the intermediary hosts in the transmission of malarial fever; this is particularly true when the malaria-transmitting anopheles have different breeding habits from those which are not responsible for the spread of malarial fever. Various observers have published notes on the breeding habits of anopheles. It may be said that there is as much selection of breeding-places by anopheles as there is selection of feeding-grounds by fish. Trout, salmon and bullheads have their analogues among anopheles larvæ, some requiring fresh aerated water, or water containing much green algæ; others select tree-holes and the recesses of epiphytic tree-plants, such as bromelias, as breeding-places; while others preferring fresh aerated water are so adaptable that they will breed in brackish water, containing half its volume of sea-water; some species require plenty of sunlight, while other sylvan species prefer shady pools in which chlorophyl-bearing algæ are relatively absent.

The identification of mosquitoes used in these experiments has been corrected or verified by Mr. A. H. Jennings, and his identifications are being checked in the Bureau of Entomology, U. S. Department of Agriculture, Washington.[1]

Notes on the species of anophelines in the Canal Zone, their relative numbers and breeding habits, were published by Mr. August Busck of the U. S. Bureau of Entomology in May, 1908. Eleven species of anophelines have been collected in the Canal Zone. Of these eleven species the three commonest ones are *A. albimanus, A. pseudopunctipennis* and *A. malefactor. A. albimanus* is much the commonest anopheles in the Canal Zone and is the one oftenest taken in quarters and barracks, although the proportion of one species to another varies somewhat with the season and locality. In certain villages only *A. albimanus* is taken in barracks,

1. Dr. H. G. Dyer has since verified all our identifications save one rubbed specimen which is probably *A. apicimacula.*

while in other villages from 5 to 10 per cent. will be *A. pseudopunctipennis,* and at Ancon during October, 1908, 27.5 per cent. were *A. malefactor* and 72.5 per cent. were *A. albimanus.* Tree-breeding species are rarely encountered and I know of no instance in which specimens of this species have been taken in quarters. The female anopheles visits quarters to obtain blood, which is necessary for the development of her ova. When specimens of *A. albimanus* (males and females), placed in a large comfortable breeding-jar with sufficient vegetable food, such as dates and bananas, were permitted to pair, the ovaries of the females showed no development within fifteen days.

The experiments were begun in October, 1908, during the rainy season and discontinued in February, 1909, during the dry season. Mosquito larvæ were collected by sanitary inspectors at various places in the Canal Zone. On being received at the laboratory, larvæ and algæ were placed in moist jars on a table in front of a window with an eastern exposure so that they got direct sunlight for an hour or two in the morning. Predaceous larvæ were killed and removed from the breeding-tanks. A preliminary difficulty was experienced in the gradual fouling the water of the tank; this was obviated in a very simple way by passing air bubbles through the water with a Paquelin cautery bulb. In this way the water was kept fresh and the larvæ remained vigorous. After the larvæ had pupated they were placed in breeding-out tubes plugged with cotton, and after twenty-four or forty-eight hours the newly emerged mosquitoes were transferred to jars made of lantern chimneys, covered with crinoline gauze, where they might pair and be kept for biting. Females would bite twenty-four to forty-eight hours after emerging; frequently on successive and always on alternate nights for a period of at least fifteen days. The species of anopheles used were *A. albimanus, A. pseudopunctipennis, A. franciscanus, A. argyritarsis, A. malefactor* and *A. apicimacula.* These mosquitoes fall into three groups: the white hind-footed group; the uniformly colored group; and the spotted-legged group.

A series of bitings were conducted on suitable infected patients who were carrying estivoautumnal or tertian gametes in their peripheral blood, using four varieties of mosquitoes. Out of several hundred mosquitoes used in the biting experiments, 100 mosquitoes

were dissected, and of these 70.8 per cent. of *A. albimanus* became infected; 12.9 per cent. of *A. pseudopunctipennis* became infected, and none of *A. malefactor* (17 mosquitoes used) became infected, although several of the latter were purposely placed in jars with *A. albimanus* and bit at the same time persons from whom the specimens of *A. albimanus* became infected.

It is concluded from this series of biting experiments that *A. albimanus,* the common white hind-footed mosquito—an extremely hardy, vigorous, rapidly developing, adaptable mosquito—is the transmitter of estivo-autumnal and of tertian malarial fever in the Canal Zone at this time. Specimens of this species infected with tertian parasites became infective between nine and eleven and a half days after the first feeding. When infected by estivoautumnal parasites, sporozoites appeared in the salivary glands as early as the eleventh day in some mosquitoes and later than twelve and a half days in others. *A. malefactor* is not concerned in the transmission of malarial fever in the Canal Zone at this time. *A. pseudopunctipennis* is only slightly concerned in the transmission of malarial fever; this appears not only from the fact that only four out of thirty-one mosquitoes under the most favorable artificial conditions became infected, but from the additional fact that relatively few specimens are taken in quarters at the present time.

The English investigators appear to have made no attempt to determine the limits of infectiousness of man in malarial fever. To me this appeared to be, if possible, worth determining, because in the discharge of patients from the hospital it would be manifestly unwise to turn a patient out, although free from symptoms, yet with gametes enough in his peripheral blood to infect every susceptible anopheles mosquito that might bite him. Efforts to determine the limit of infectiousness of man were made in the following way:

In the 47 biting experiments, several blood preparations were taken fresh and for staining, at the time of the biting. Differential counts of leucocytes and the ratio between crescents and leucocytes were determined the next morning. Leucocyte counts were occasionally made at the time the blood was taken so as to check this method of estimating the crescents. In this way it was always known how many crescents were in the peripheral blood per c.mm. at the time the mosquito was in-

fected. The next step was to weigh a number of mosquitoes before and after biting in order to estimate the amount of blood retained by a mosquito. In this way the approximate number of gametes ingested could be calculated. Again, smears were made from the midgut of mosquitoes that had a few minutes before bitten a person whose blood contained numerous gametes. From this it was found that about one-half the gametes were phagocyted by polymorphonuclear leucocytes and thus destroyed. Finally, mosquitoes which had fed but once on patients whose crescents per leucocytes had been estimated were fed subsequently on dates, and on dissection of these mosquitoes the probable limit of infectiousness was determined as being near one gamete per 500 leucocytes. From these observations, I am confident that a patient with more than one crescent for every 500 leucocytes, or 12 crescents per c.mm., is infective, and it follows that such an individual should not be discharged from treatment in that condition or should be warned or required to continue treatment. Such a person is a gamete-carrier and is a menace to a malarial community whenever susceptible anopheles have access to him.

The effect of quinin administration on the number of gametes in the peripheral blood was studied in a number of patients suffering from estivoautumnal and tertian fever by the administration of 30 grains quinin daily, either with or without iron and arsenic, and by withholding quinin and merely requiring rest from labor. In estivoautumnal infections, when there were crescents in the blood, the number was reduced by quinin, 30 grains daily, at the following rates. For example, in Experiment 17 there were:

9 crescents per 100 leucocytes on December 26
5 crescents per 100 leucocytes on December 27
5 crescents per 100 leucocytes on December 28
4 crescents per 100 leucocytes on December 30
1 crescent per 100 leucocytes on December 31
0 crescents per 100 leucocytes on January 2
0 crescents per 100 leucocytes on January 13

In Experiment 20 the crescents were reduced from 67 per 100 leucocytes to one per 200 leucocytes in twenty-five days.

In Experiment 41 crescents were reduced from 92 per 100 leucocytes to one per 100 leucocytes in fifteen days; while, on the other hand, in Experiment 6, when

quinin was withheld, crescents remained constantly in the peripheral blood for twenty-three days, there being 16 crescents per 100 leucocytes on admission and 20 crescents per 100 leucocytes at the end of the period.

From this it is concluded that generous daily doses of quinin, grains 10 t. i. d., will reduce the sexual forms of the estivoautumnal parasite to a non-infectious minimum in from a few days to two or three weeks, depending on the severity of the infection.

In tertian malarial fever there is never an abundance of gametes in the peripheral blood after the disappearance of the asexual forms such as is seen in estivoautumnal infections; besides, all forms of the tertian parasite disappear from the peripheral blood within two or three days under quinin treatment and occasionally disappear when quinin is withheld.

A curious relation was observed between the average number of days that gametes remained in the blood in estivoautumnal and tertian infections when under quinin treatment, the number being about ten in estivoautumnal and three in tertian, and the average proportion of the incidence of estivoautumnal to tertian malarial fever in the Canal Zone. This might be interpreted as indicating that the proportion of cases of estivoautumnal to tertian malaria depends on the number of days that gametes are in the peripheral blood in numbers sufficient to infect *A. albimanus,* which is the host for both forms of the parasite.

The infection of *A. albimanus* and the development of malarial parasites in her midgut is not interfered with by quinin when such an infected mosquito feeds daily or on alternate days for fifteen days on patients who are receiving 30 grains of quinin in solution daily. In these instances the zygotes mature and sporozoites reach the salivary glands in the usual period.

Observations on latent malaria were made during the past dry season in several villages where the malarial sick-rate did not fall to zero and where no anopheles were breeding. On a systematic blood examination of laborers and their families, I found that 10 per cent. of men who were at work without symptoms had parasites. Three per cent. of the cases of latent malaria were of the estivoautumnal variety and 7 per cent. of the tertian variety. Among the families of Spanish and West Indian laborers the amount of latent malaria was 30 per cent. A few cases of fever would arise from this

source every week, probably following some loss of resistance caused by dissipation, wetting or overheating. It is this latent malaria in every tropical community which contributes largely to the preservation of the malarial parasite and to the infection of anopheles when, after the onset of the rainy season, mosquitoes have begun to breed in numbers.

The theories of Mannaberg and Craig explain the persistence of latent malaria by the process of conjugation of two ring forms within a red blood corpuscle. During examinations of the blood, in cases of latent malaria, I have frequently seen estivoautumnal crescents which had apparently been formed by the fusion or conjugation of two ring forms, or of two pigmented forms, but I have also very frequently seen in estivoautumnal and tertian malaria red blood cells infected by two or more ring forms which have gone on to maturity without conjugation. Sometimes these contiguous parasites were of the same age, but oftener one would be a little older.

Quinin administered in latent malaria appears to destroy the ring forms only and prevents the possibility of their development into gametes. The gametes in the blood stream are probably phagocyted by splenic and hepatic endothelial cells and are not influenced by quinin directly.

The points I wish to emphasize are these:

A. albimanus, the common white hind-footed anopheles, is the host for estivoautumnal and tertian malarial parasites in the Canal Zone at this time.

A. malefactor, notwithstanding its name, does not transmit malarial fever.

A. pseudopunctipennis is only slightly concerned in the transmission of malarial fever.

In the efforts at mosquito destruction, the extermination of *A. albimanus* is of paramount importance.[2]

Patients having crescents or tertian gametes in their peripheral blood should not be discharged from the hospital, nor should treatment be discontinued until gametes have been reduced to a non-infectious minimum. The destruction and prevention of development of the sexual parasites in man is of great importance

2. I have since determined that *A. tarsimaculata* also transmits estivoautumnal parasites. This mosquito belongs to the white-hind-footed group and has recently been breeding in rather large numbers in brackish water near Colon.

and may be accomplished (1) by appropriate quinin treatment of all gamete-carriers entering the hospital; (2) by occasional quinin treatment to destroy latent malaria; (3) by the periodical blood examination of laborers in quarters where there is a high malarial rate for the detection of gamete-carriers and latent malaria in order to carry out appropriate treatment.

Thirty grains of quinin sulphate in solution daily is an efficient dose for the purpose required.

Ancon Hospital.

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